British Journal of Pharmacology (2000) 131, 1546–1552

www.nature.com/bjp

Platelet-derived growth factor causes endothelium-independent relaxation of rabbit mesenteric artery *via* the release of a prostanoid

[1,3]Hideyuki Yamawaki, [2]Koichi Sato, [1]Masatoshi Hori, *,[1]Hiroshi Ozaki & [1]Hideaki Karaki

[1]Department of Veterinary Pharmacology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan and [2]Radio Isotope Center, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

1 Recent evidence has indicated that the mitogen platelet-derived growth factor (PDGF) can act acutely to regulate arterial tone. In this study we demonstrate that the BB isoform of PDGF (PDGF-BB) can cause endothelium-independent relaxation of rabbit isolated mesenteric arteries.

2 In endothelium-denuded arteries, PDGF-BB (40 pM – 8.0 nM) caused concentration-dependent relaxation of noradrenaline-induced tone. The relaxation to PDGF-BB was abolished by a cyclo-oxygenase inhibitor, indomethacin (10 μM) and by the PDGF receptor-associated tyrosine kinase inhibitor, tyrphostin AG1295 (50 μM), but not by N^G-monomethyl-L-arginine (L-NMMA, 200 μM), an inhibitor of nitric oxide (NO) synthase.

3 PDGF-BB (4.0 nM) significantly increased the release of prostacyclin (PGI_2) in endothelium-denuded arteries. Exogenously applied iloprost (1 μM), a stable analogue of PGI_2, relaxed the endothelium-denuded artery. PDGF-BB (4.0 nM) significantly increased the cyclic AMP content.

4 In the absence of Ca^{2+}, PDGF-BB (4.0 nM) failed to relax the artery, and the release of PGI_2 was almost completely suppressed. In addition, the release of PGI_2 by PDGF-BB (4.0 nM) was significantly reduced in the presence of endothelium. The effect of endothelium was eliminated by L-NMMA (200 μM) and PGI_2 release increased.

5 These data indicate that in rabbit mesenteric arteries, PDGF-BB can evoke endothelium-independent relaxation by stimulating the synthesis of PGI_2. The PDGF-BB-induced prostaglandin synthesis is dependent on both Ca^{2+} and tyrosine phosphorylation of the PDGF receptor, and is attenuated by endothelium-derived NO.

British Journal of Pharmacology (2000) **131,** 1546–1552

Keywords: PDGF; rabbit mesenteric artery; prostaglandin; relaxation; smooth muscle; endothelium; calcium; nitric oxide

Abbreviations: COX, cyclo-oxygenase; EDTA, ethylenediaminetetra-acetic acid; EGTA, O, O′-bis (2-aminoethyl) ethyleneglycol-N,N,N′,N′-tetra-acetic acid; EIA, enzyme immunoassay; IBMX, 3-isobutyl-1-methylxanthine; L-NMMA, N^G-monomethyl-L-arginine; NO, nitric oxide; PDGF, platelet-derived growth factor; PG, prostaglandin; PGI_2, prostacyclin; PSS, physiological salt solution; TCA, trichloroacetic acid

Introduction

Platelet-derived growth factor (PDGF) is a potent mitogen which plays a primary role in the vascular response to injury and the development of atherosclerosis by stimulating cell proliferation and migration (Ross, 1993; Ross *et al*., 1986). PDGF is a dimer protein composed of two homologous polypeptide chains (A and B), and two receptors for PDGF (α and β) bind these two PDGF chains with different affinities (Claesson-Welsh, 1994). The α-receptor binds both PDGF-A and -B chains with similar affinity, whereas the β-receptor binds only the PDGF-B chain. Binding of PDGF chains to the specific receptors can induce the receptor dimerization and subsequent autophosphorylation at sites of tyrosine residues, which appears to be an important process in the biological actions of PDGF (Claesson-Welsh, 1994). There is accumulating evidence indicating that, in addition to mitogenic actions, PDGF has acute effects on blood vessels to change their contractility. For example, it has been shown that PDGF causes a contraction in smooth muscle of the rat aorta (Berk *et al*., 1986; Cunningham *et al*., 1992), rabbit ear artery (Hughes, 1995), and rabbit aorta (deBlois *et al*., 1992). It has been demonstrated that the contractile effect of PDGF is associated with the activation of receptor tyrosine kinase (Hughes, 1995; Sauro & Thomas, 1993) and the increase in the cytosolic Ca^{2+} concentration in smooth muscle (Berk *et al*., 1986; Hughes, 1995). In contrast, it has also been reported that PDGF causes relaxation of smooth muscle in the rat aorta and mesenteric superior and resistance artery which is endothelium-dependent (Cunningham *et al*., 1992; Ikeda *et al*., 1997; Takase *et al*., 1999). The endothelium-dependent relaxation has been shown to be mediated by endothelial-derived nitric oxide (NO; Ikeda *et al*., 1997). Despite the accumulating evidence, the precise mechanisms of the acute effects of PDGF on blood vessels are not fully understood. In the present study, we examined the mechanism of acute effects of PDGF on contractile responses in isolated rabbit superior mesenteric artery and show that PDGF can induce an endothelium-independent vascular relaxation *via* the release of vasodilator prostaglandin.

*Author for correspondence: E-mail: aozaki@mail.ecc.u-tokyo.ac.jp,
[3]Current address: Center for Cardiovascular Research, University of Rochester, 601 Elmwood Ave, Box 679, Rochester, New York 14642, U.S.A.

Methods

Tissue preparation

Male Japanese White rabbits (2–3 kg) were euthanized by stunning and exsanguination. The main branches of the superior mesenteric arteries (approximately 0.4 mm in internal diameter) were isolated. After removal of fat and adventitia, each artery was cut into rings approximately 2 mm wide for the measurement of muscle tension. The endothelium was removed by rubbing the intimal surface with the flat face of a pair of forceps. Removal of the endothelium was confirmed by the lack of relaxation induced by carbamylcholine chloride (carbachol, 1 μM). Animal care and treatment were conducted in conformity with institutional guidelines of The University of Tokyo.

Measurement of muscle tension

The arterial rings were placed in normal physiological salt solution (PSS), which contained (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1.0, $NaHCO_3$ 23.8, glucose 5.5, and ethylenediaminetetraacetic acid (EDTA) 1 μM. The Ca^{2+}-free solution was prepared by excluding $CaCl_2$ and adding O, O′-bis (2-aminoethyl) ethyleneglycol-N,N,N′,N′-tetra-acetic acid (EGTA, 2 mM). The high-K^+ (65.4 mM) solution was prepared by replacing NaCl with equimolar KCl. These solutions were saturated with a 95% O_2–5% CO_2 mixture at 37°C and pH 7.4. Muscle tension was recorded isometrically with a force-displacement transducer (Orientec, Japan). Each muscle ring was mounted in a 2-ml organ bath under a resting tension of 10 mN. After 30 mins equilibration, each ring was repeatedly exposed to high-K^+ solution until the responses became stable (60–90 min). Concentration-response curves were obtained by the cumulative application of PDGF isoforms (AA and BB) to arteries pre-contracted with noradrenaline (10 μM). The magnitude of the contraction to noradrenaline was $90.3 \pm 1.0\%$ ($n=14$) of the maximum contraction induced by 100 μM noradrenaline (Yamawaki *et al*., 2000). In some experiments, a maximally effective concentration of endothelin-1 (100 nM; Iwasaki *et al*., 1999) was used to induce pre-constriction. Data are shown as per cent relaxations of the steady-state pre-constriction.

Measurement of 6-keto-prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$)

Bolus doses of PDGF-BB (0.4, 2.0 or 4.0 nM) were applied to tissues pre-contracted with noradrenaline (10 μM) and tension continuously monitored. When the PDGF-induced relaxation reached a steady state (approximately 15 min after the PDGF-treatment), the incubation solution (50 μl) was collected. In the control experiments, vehicle (distilled water) was applied instead of PDGF-BB. The concentration of 6-keto-$PGF_{1\alpha}$ (a stable product of prostacyclin, PGI_2) in the incubation solution was measured with enzyme immunoassay (EIA) kits according to the manufacturer's protocol (RPN 221, Amersham Life Sciences, U.S.A.). The measurable range of 6-keto-$PGF_{1\alpha}$ by this method was 0.5–64 pg. The concentration of prostaglandin is expressed as pg mg^{-1} tissue wet weight.

Measurement of cyclic AMP

Endothelium-denuded arterial rings were equilibrated in PSS aerated with a 95% O_2–5% CO_2 mixture at 37°C for 45 min without resting tension before the start of experiments. After pretreatment with noradrenaline (10 μM) for 10 min, arterial rings were incubated with PDGF-BB (4.0 nM) for 20 min in the presence of phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX, 500 μM). In the control experiments, vehicle (distilled water) was added instead of PDGF-BB. After the incubation, the arterial preparations were quickly frozen with liquid nitrogen and homogenized in ice-cold 6% trichloroacetic acid (TCA) with a Polytron homogenizer. After centrifugation at $1500 \times g$ for 15 min, TCA in the supernatant was removed by washing with water-saturated ether, and cyclic AMP was measured with EIA kits according to the manufacturer's protocol (RPN 225, Amersham Life Sciences, U.S.A.). The measurable range of cyclic AMP by this method was 12.5–3200 fmol. The protein concentration of each preparation was determined by the method described by Bradford (1976), and the content of cyclic AMP was expressed as pmol mg^{-1} protein.

Chemicals

The chemicals used were as follows: indomethacin, IBMX (Sigma, U.S.A.), EDTA and EGTA (Dojindo Laboratories, Japan), NS-398 (Cayman Chemical, U.S.A.), 6-keto-$PGF_{1\alpha}$ EIA system, cyclic AMP EIA system (Amersham Life Sciences, U.S.A.), noradrenaline, N^G-monomethyl-L-arginine (L-NMMA), caffeine, carbachol (Wako Pure Chemical, Japan), recombinant human PDGF-AA, PDGF-BB (Austral Biologicals, U.S.A.), endothelin-1 (Peptide Institute, Japan), tyrphostin AG1295 (Calbiochem, U.S.A.), and iloprost (generous gifts from Eisai Co., Japan).

Statistical analysis

Results are expressed as mean ± s.e.mean. Statistical evaluation of the data was performed by unpaired Student's *t*-test for comparisons between two groups and by one-way analysis of variance (ANOVA) followed by Dunnett's test for comparisons between more than two groups. A value of $P<0.05$ was taken as significant. All pD_2 values were calculated as the $-\log_{10}EC_{50}$ (i.e. that concentration at which the half maximal effect occurred) by sigmoidal curve fitting.

Results

Effects of PDGF on rabbit mesenteric arterial smooth muscle

In endothelium-denuded segments of rabbit mesenteric artery, cumulative additions of PDGF-BB (2.0 pM–8.0 nM) did not change the resting tension ($n=5$). However in endothelium-denuded tissues pre-contracted with noradrenaline (10 μM), cumulative additions of PDGF-BB (40 pM–8.0 nM) induced a concentration-dependent relaxation (Figure 1A,B) with a pD_2 value of 9.08 ± 0.13 ($n=11$). The maximal relaxation caused by 8.0 nM PDGF-BB was $87.0 \pm 3.4\%$ ($n=11$). The 4.0 nM PDGF-BB-induced relaxation started after a delay of 2.8 ± 0.4 min and reached a peak at 15.9 ± 0.5 min ($n=24$).

In the endothelium-denuded arteries, AA isoform of PDGF (PDGF-AA, 2.0 pM–8.0 nM) did not change the resting tension ($n=6$). In contrast, the cumulative addition of PDGF-AA (40 pM–8.0 nM) induced a concentration-dependent relaxation of noradrenaline-induced tone (Figure 1B) with a pD_2 value of 9.08 ± 0.38 ($n=10$). The maximal

relaxation caused by 4.0 nM PDGF-AA (56.8±9.0%) was significantly less than that of PDGF-BB ($P<0.01$).

Effects of inhibitors on the PDGF-induced relaxation

Pretreatment of endothelium-denuded rabbit mesenteric arteries with a NO synthase inhibitor, L-NMMA (200 μM, added 30 min before the addition of noradrenaline), had no effect on the PDGF-BB (40 pM–4.0 nM)-induced relaxation of noradrenaline-induced pre-constriction (Figure 2, $n=6$). In contrast, indomethacin (10 μM, 30 min), a non-selective inhibitor of cyclo-oxygenase (COX), completely abolished the PDGF-BB-induced relaxation (Figure 2, $n=6$). On the other hand, NS-398 (1 μM, 30 min), a specific inhibitor of the inducible isoform of COX (COX-2), had no effects on the PDGF-BB-induced relaxation (Figure 2, $n=10$). In addition, tyrphostin AG1295 (50 μM, 15 min), a specific inhibitor of PDGF receptor-associated tyrosine kinase, almost completely abolished the PDGF-BB-induced relaxation (Figure 2, $n=7$). None of these inhibitors altered the amplitude of the contraction to noradrenaline (10 μM; $n=6-10$).

Release of prostaglandin from the arterial tissue

In control endothelium-denuded rabbit mesenteric arteries, the amount of PGI_2 released into the incubation solution was 8.5±2.5 pg mg^{-1} wet weight ($n=6$). In the arteries stimulated with PDGF-BB (0.4, 2.0, or 4.0 nM), the release of PGI_2 increased in a concentration-dependent manner (Figure 3, $n=6-7$). Treatment with indomethacin (10 μM, added 30 min before the addition of noradrenaline) completely suppressed the 4.0 nM PDGF-BB-induced PGI_2 release (Figure 3, $n=6$, $P<0.01$).

Effects of PDGF on cyclic AMP content in smooth muscle

In the absence of PDGF-BB, the content of cyclic AMP in the endothelium-denuded rabbit mesenteric arteries was 46.1±8.7 pmol mg^{-1} protein ($n=11$). In the arteries treated with PDGF-BB (4.0 nM) for 20 min, the content of cyclic AMP increased significantly to 92.3±20.0 pmol mg^{-1} protein ($n=11$, $P<0.05$).

Role of Ca^{2+} on the PDGF-induced relaxation

It has been shown that endothelin-1 but not noradrenaline causes a considerable contraction of vascular smooth muscle, even in the absence of external Ca^{2+} (Hori *et al*., 1992). Therefore, we used endothelin-1 as a vasoconstrictor to examine the role of Ca^{2+} on the PDGF-induced relaxation. In normal PSS, maximally effective concentration of endothelin-1 (100 nM) caused a transient contraction followed by a sustained contraction in the endothelium-denuded rabbit mesenteric artery (Figure 4A). The addition of PDGF-BB (4.0 nM) inhibited the endothelin-1-induced sustained contraction by 77.0±3.7% ($n=10$). This value was not significantly different from the effect of 4.0 nM PDGF-BB on noradrenaline-induced tone (63.4±3.8%, $n=39$). In Ca^{2+}-free PSS with EGTA (2 mM), the addition of caffeine

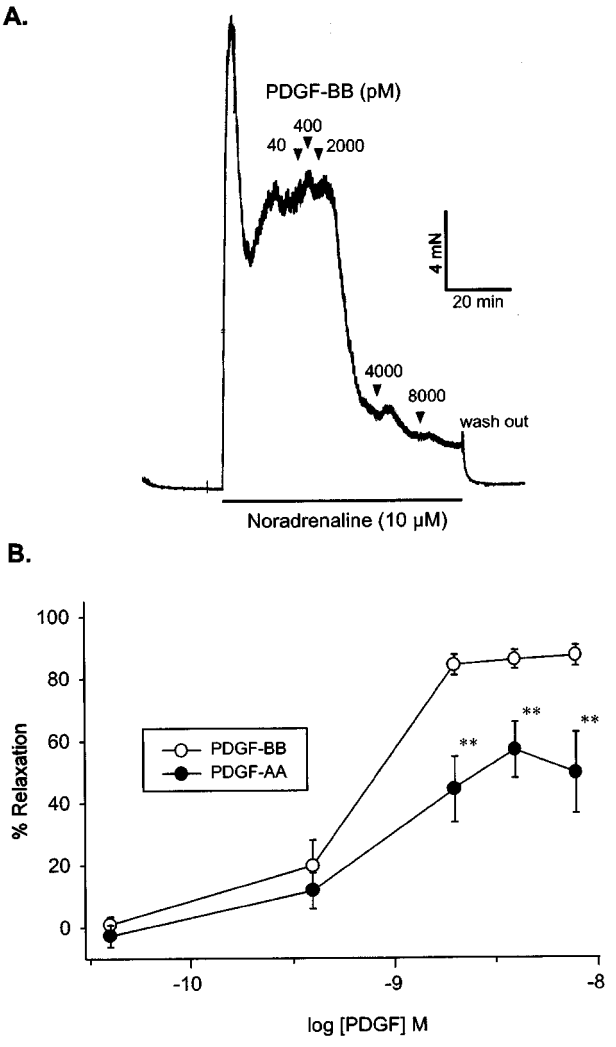

Figure 1 (A) Representative recording of the relaxant effect of PDGF-BB (40 pM–8.0 nM) in an endothelium-denuded artery pre-contracted with noradrenaline (10 μM). PDGF-BB was cumulatively added after the contraction induced by noradrenaline had reached a steady state. (B) Concentration-response relationship for the relaxant effect of PDGF-AA and -BB (40 pM–8.0 nM) on the noradrenaline (10 μM)-induced sustained contraction in the endothelium-denuded rabbit mesenteric arteries. 100% represents the steady-state pre-constriction. Results are expressed as mean±s.e.mean of 10–11 experiments. **Significantly different from PDGF-BB with $P<0.01$.

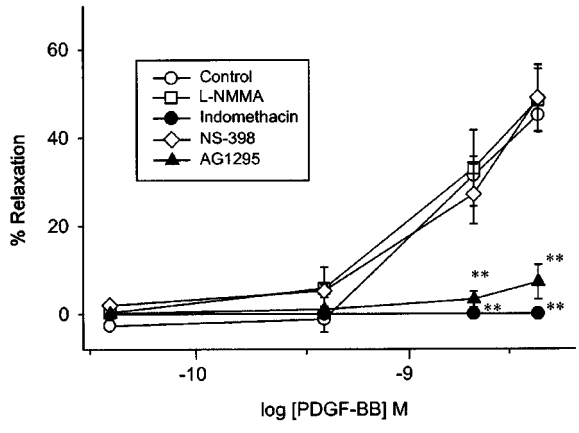

Figure 2 Concentration-relaxation relationships for PDGF-BB in the presence of L-NMMA (200 μM), indomethacin (10 μM), NS-398 (1 μM), or tyrphostin AG1295 (50 μM). PDGF-BB (40 pM–4.0 nM) was cumulatively added after the contraction induced by noradrenaline (10 μM) had reached a steady state. L-NMMA, indomethacin, and NS-398 were added 30 min before whereas tyrphostin AG1295 was added 15 min before the addition of noradrenaline. 100% represents the steady-state pre-constriction. Results are expressed as mean±s.e.mean of 6–10 experiments. **Significantly different from the relaxation in the absence of inhibitor (control) with $P<0.01$.

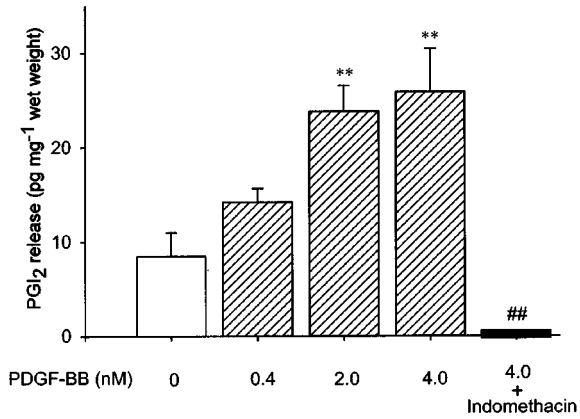

Figure 3 Concentration-dependent effects of PDGF-BB on the release of PGI_2 (measured as 6-keto-$PGF_{1\alpha}$) in the endothelium-denuded rabbit mesenteric arteries. PDGF-BB (0.4, 2.0 or 4.0 nM) was applied to the arteries pre-contracted with noradrenaline (10 μM). When the PDGF-induced relaxation reached a steady-state (approximately 15 min after the PDGF-treatment), the incubation solution (50 μl) was collected. PGI_2 in the incubation solution was measured by enzyme immunoassay (EIA), and the amounts are expressed as pg mg^{-1} tissue wet weight. Inhibitory effect of indomethacin (10 μM, added 30 min before the addition of noradrenaline) on the PDGF-BB (4.0 nM)-induced PGI_2 release was also examined. Results are expressed as mean$\pm$s.e.mean of 6–7 experiments. **Significantly different from the PGI_2 release in the absence of PDGF-BB with $P<0.01$. ##Significantly different from the 4.0 nM PDGF-BB-induced PGI_2 release with $P<0.01$.

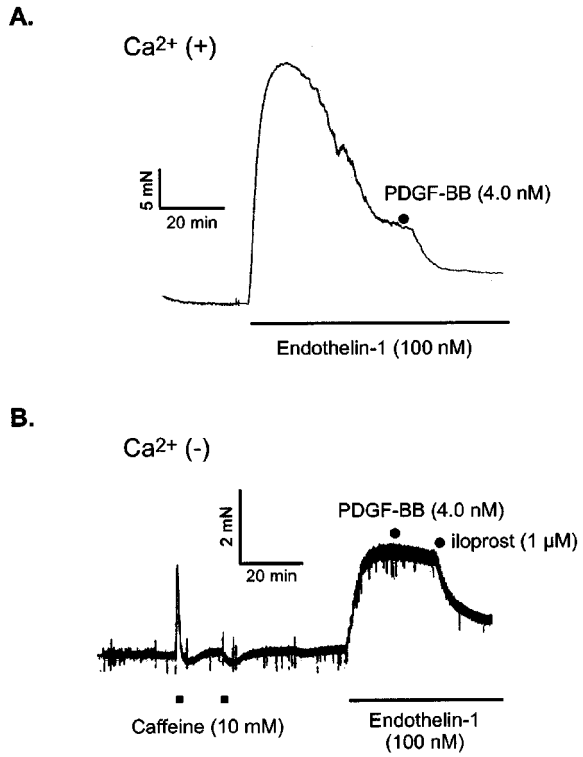

Figure 4 Representative recordings of the effects of PDGF-BB (4.0 nM) on the endothelin-1 (100 nM)-induced sustained contraction in normal PSS (A) or in Ca^{2+}-free PSS (B).

(10 mM), an activator of Ca^{2+} channel on the sarcoplasmic reticulum (SR), caused a transient contraction in the endothelium-denuded rabbit mesenteric artery (Figure 4B). The second application of caffeine did not induce any contraction, suggesting a depletion of SR Ca^{2+}. After the washout of caffeine with Ca^{2+}-free PSS, the addition of endothelin-1 (100 nM) caused a sustained contraction which was not altered by PDGF-BB (4.0 nM; $n=10$). In contrast, iloprost (1 μM), a stable analogue of PGI_2, inhibited the contraction by 61.6$\pm$3.6% ($n=5$). In the absence of Ca^{2+}, the release of PGI_2 by PDGF-BB (4.0 nM) was almost completely suppressed (1.5$\pm$0.9 pg mg^{-1} wet weight, $n=4$, $P<0.01$).

Effects of endothelium on the PDGF-induced relaxation and prostaglandin release

In the endothelium-intact rabbit mesenteric arteries, the cumulative addition of PDGF-BB (40 pM–4.0 nM) also caused a concentration-dependent relaxation of the 10 μM noradrenaline-induced contraction (Figure 5A, $n=10$). However, the relaxation due to 2.0 or 4.0 nM PDGF-BB was significantly smaller in the endothelium-intact arteries ($P<0.05$).

In the absence of PDGF-BB, the amount of PGI_2 released from the endothelium-intact rabbit mesenteric arteries was 3.0$\pm$0.9 pg mg^{-1} wet weight ($n=6$, Figure 5B). This value was similar to that in the control endothelium-denuded arteries (3.4$\pm$1.6 pg mg^{-1} wet weight, $n=5$, Figure 5B). In endothelium-intact arteries stimulated with PDGF-BB (4.0 nM), release of PGI_2 increased significantly to 11.9$\pm$1.4 pg mg^{-1} wet weight (Figure 5B, $n=6$, $P<0.01$ from control endothelium-intact arteries). However, this value was significantly smaller than in the endothelium-denuded arteries stimulated with 4.0 nM PDGF-BB (20.1$\pm$2.4 pg mg^{-1}, wet weight, $n=6$, $P<0.05$, Figure 5B). In the endothelium-intact arteries pretreated with L-NMMA (200 μM, added 30 min before the addition of noradrenaline), release of PGI_2 by PDGF-BB (4.0 nM) increased to the level similar to that in the endothelium-denuded arteries (19.6$\pm$2.6 pg mg^{-1} wet weight, $n=6$, Figure 5B).

Discussion

In the present study, we have shown that PDGF (-AA and -BB) causes relaxation of the endothelium-denuded rabbit mesenteric artery pre-contracted with noradrenaline. This is the first report of PDGF causing relaxation of vascular smooth muscle in an endothelium-independent manner. It was also noted that PDGF (-AA and -BB) (2.0 pM–8.0 nM) does not cause a contraction in the rabbit mesenteric artery. In agreement with the present results, Takase *et al.* (1999) have reported that all isoforms of PDGF (PDGF-AA, -BB, and -AB) (0.1–10 nM) had no contractile effect in rat mesenteric resistance artery. In other studies, however, it has been reported that isoforms of PDGF cause a contraction in the rat aorta (Berk *et al.*, 1986; Cunningham *et al.*, 1992), rabbit ear artery (Hughes, 1995), and rabbit aorta (deBlois *et al.*, 1992). The EC_{50} value for rabbit aorta was around 1 nM whereas the values for rat aorta and rabbit ear artery were much lower (around 30 pM). These results suggest the presence of tissue differences in the sensitivity to the contractile effect of PDGF.

It has also been demonstrated that isoforms of PDGF cause an endothelium-dependent NO-mediated relaxation in rat aorta (Cunningham *et al.*, 1992; Ikeda *et al.*, 1997) and rat superior (Ikeda *et al.*, 1997) and resistance mesenteric artery (Takase *et al.*, 1999). In the rat aorta that EC_{50} value for the endothelium-dependent relaxant effect of PDGF (around 100 pM) was higher than that for the contractile effect (around 30 pM). In the present experiments, we

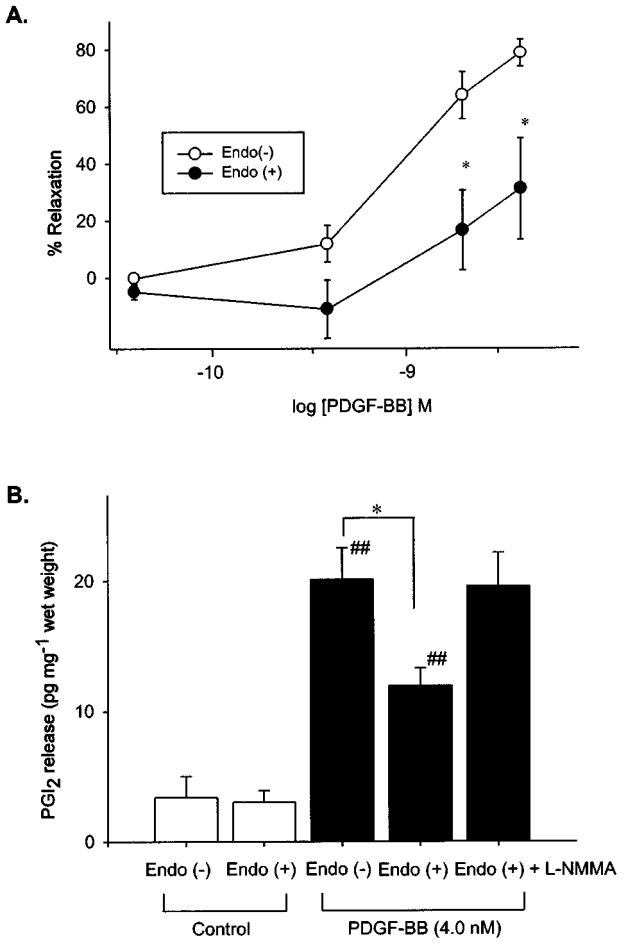

Figure 5 (A) Concentration-relaxation relationship for PDGF-BB on the noradrenaline-induced sustained contraction in the endothelium-denuded or endothelium-intact rabbit mesenteric arteries. PDGF-BB (40 pM–4.0 nM) was cumulatively added after the contraction induced by noradrenaline (10 μM) had reached a steady state. 100% represents the steady-state pre-constriction. Results are expressed as mean$\pm$s.e.mean of 8–10 experiments. *Significantly different with $P<0.05$. (B) Release of PGI_2 from the endothelium-denuded and endothelium-intact rabbit mesenteric arteries stimulated without or with PDGF-BB. PDGF-BB (4.0 nM) was applied to the muscle pre-contracted with noradrenaline (10 μM). When the PDGF-induced relaxation reached a steady state (approximately 15 min after the PDGF-treatment), the incubation solution (50 μl) was collected. PGI_2 in the incubation solution was measured by enzyme immunoassay (EIA), and the amounts are expressed as pg mg^{-1} tissue wet weight. Effects of L-NMMA (200 μM, added 30 min before the addition of noradrenaline) on the PDGF-BB (4.0 nM)-induced PGI_2 release were also examined in the endothelium-intact arteries. Results are expressed as mean$\pm$s.e.mean of 5–6 experiments. ##Significantly different from each control with $P<0.01$. *Significantly different with $P<0.05$.

examined the effects of wide concentration range (2.0 pM–4.0 nM) of PDGF (-AA and -BB) and found that PDGF did not relax the endothelium-intact artery in the presence of indomethacin ($n=4$). This result suggests that PDGF cannot cause an endothelium-dependent NO-mediated relaxation in the rabbit mesenteric artery.

As isoforms of PDGF other than PDGF-BB had a small, if any, effect on endothelium-dependent relaxation in the rat aorta (Cunningham *et al*., 1992; Ikeda *et al*., 1997) and rat resistance mesenteric artery (Takase *et al*., 1999), presence of the β-receptor in the endothelium was predicted. In the present study, both PDGF-AA and PDGF-BB caused a relaxation in the endothelium-denuded rabbit mesenteric artery, although the relaxant effect of PDGF-BB was larger than that of PDGF-AA. Although these observations suggest that both the PDGF α- and β-receptors may be responsible for the endothelium-independent relaxant effects, molecular biological experiments are necessary to identify the exact receptor subtypes.

It is well documented that the tyrosine phosphorylation of the PDGF receptor is a prerequisite for exerting the biological action of PDGF (Claesson-Welsh, 1994) and that contractile effect of PDGF is associated with the tyrosine kinase activation (Hughes, 1995; Sauro & Thomas, 1993). The present results indicate that tyrphostin AG1295, a highly selective inhibitor of PDGF receptor tyrosine kinase (Kovalenko *et al*., 1994; Levitzki & Gazit, 1995), almost completely suppressed the PDGF-BB-induced endothelium-independent relaxation. This result suggests that endothelium-independent relaxation caused by PDGF is associated with the tyrosine phosphorylation of the PDGF receptor.

The PDGF-BB-induced relaxation was not affected by pretreatment with an NO synthase inhibitor, L-NMMA, suggesting that NO is not involved in the PDGF-induced relaxation. However, treatment with indomethacin, an inhibitor of COX, completely abolished the PDGF-BB-induced relaxation. In addition, PDGF-BB significantly increased the release of PGI_2 from the arteries. Furthermore, exogenously applied iloprost, a stable analogue of PGI_2, to the endothelium-denuded rabbit mesenteric artery inhibited the noradrenaline-induced contraction (the maximal relaxation elicited by 1 μM iloprost was 75.0$\pm$7.6%, $n=4$). These results suggest that PDGF-BB-induced relaxation is due to the release of PGI_2. It is well known that the vasodilator prostaglandins, including PGI_2, activate adenylate cyclase to increase the cyclic AMP content in smooth muscle (Smith, 1986). We also showed that PDGF significantly increased the amount of cyclic AMP in the endothelium-denuded arteries. It has been reported that forskolin, an activator of adenylate cyclase, inhibited the noradrenaline-induced contraction in the rabbit mesenteric artery (Ito *et al*., 1993; Khan *et al*., 1993). Thus, the present results strongly suggest that PDGF-BB-induced relaxation is due to the release of PGI_2 and the subsequent stimulation of cyclic AMP synthesis.

COX is the enzyme that converts arachidonic acid to PGH_2, which can be further metabolized to prostanoids including PGI_2, PGE_2, and thromboxane A_2 (Otto & Smith, 1995). It is well recognized that COX exists in at least two distinct isoforms. COX-1 is constitutively expressed in many cell types, including vascular smooth muscle cells (Pritchard *et al*., 1994; Vinals *et al*., 1997). In contrast, COX-2 is induced by proinflammatory or mitogenic stimuli. It has recently been reported that PDGF causes an induction of COX-2 mRNA followed by the synthesis of COX-2 protein and prostanoid in cultured aortic smooth muscle cells (Rimarachin *et al*., 1994). These authors have also suggested that the induction of COX-2 mRNA by PDGF requires a lag time in which the induction peaks at 45–90 min. In contrast, the present results showed that PDGF-BB caused a relaxation that peaked at approximately 15 min and that NS-398, a selective inhibitor of COX-2 (Gierse *et al*., 1995), failed to inhibit the PDGF-induced relaxation, although indomethacin, a nonselective inhibitor of COX, completely inhibited the relaxation suggesting that the relaxant effect of PDGF-BB is mediated by COX-1.

In the present study, PDGF-induced relaxation was completely suppressed in the absence of Ca^{2+} and the release of PGI_2 by PDGF-BB was almost completely suppressed in the absence of Ca^{2+}. These results suggest that PDGF-BB cannot stimulate the synthesis of prostaglandin in the absence

of Ca^{2+} and thus fails to cause a relaxation of smooth muscle. This proposal is supported by the observation that exogenously applied iloprost, a PGI_2 analogue, which has been shown to inhibit smooth muscle contraction not only by decreasing the cytosolic Ca^{2+} levels but also by decreasing the Ca^{2+}-sensitivity of contractile elements (Ozaki *et al*., 1996), caused a relaxation of smooth muscle even in the absence of Ca^{2+}.

We further examined the influence of endothelium on the PDGF-BB-induced relaxation and found that the presence of endothelium significantly attenuated the relaxant effect of PDGF. Consistent with this result, release of PGI_2 by PDGF-BB was significantly reduced in the presence of endothelium. In cultured vascular endothelial cells, it has recently been demonstrated that NO plays an inhibitory role in bradykinin- or shear stress-induced PGI_2 synthesis (Matthews *et al*., 1995; Osanai *et al*., 2000). Although PDGF-BB does not stimulate the endothelial NO release in the rabbit mesenteric artery, previous studies have demonstrated that large amounts of NO are spontaneously released from the endothelium of the artery (Fujimoto & Itoh, 1997; Li & Kuriyama, 1993). To confirm the inhibitory effect of endothelial NO on smooth prostaglandin synthesis, we examined the effects of a NOS inhibitor on the PDGF-induced prostaglandin synthesis in the endothelium-intact arteries. Results showed that the NOS inhibitor completely restored the PGI_2 production in the endothelium-intact arteries, suggesting that the endothelium-derived NO inhibits the PDGF-induced prostaglandin synthesis in smooth muscle.

In summary we have shown for the first time that PDGF-BB causes an endothelium-independent vascular relaxation, which is mediated by the activation of COX-1 and the resulting release of PGI_2. The PDGF-BB-induced prostaglandin synthesis was found to be dependent on Ca^{2+} and tyrosine phosphorylation of the PDGF receptor, and attenuated by endothelium-derived NO.

This work was supported by Grants-in-Aid for Scientific Research from the Health Science Foundation, the Ministry of Education, Science, Sports and Culture, Japan, and Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists.

References

BERK, B.C., ALEXANDER, R.W., BROCK, T.A., GIMBRONE, JR., M.A. & WEBB, R.C. (1986). Vasoconstriction: a new activity for platelet-derived growth factor. *Science,* **232,** 87–90.

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.,* **72,** 248–254.

CLAESSON-WELSH, L. (1994). Platelet-derived growth factor receptor signals. *J. Biol. Chem.,* **269,** 32023–32026.

CUNNINGHAM, L.D., BRECHER, P. & COHEN, R.A. (1992). Platelet-derived growth factor receptors on macrovascular endothelial cells mediate relaxation via nitric oxide in rat aorta. *J. Clin. Invest.,* **89,** 878–882.

DEBLOIS, D., DRAPEAU, G., PETITCLERC, E. & MARCEAU, F. (1992). Synergism between the contractile effect of epidermal growth factor and that of des-Arg^9-bradykinin or of α-thrombin in rabbit aortic rings. *Br. J. Pharmacol.,* **105,** 959–967.

FUJIMOTO, S. & ITOH, T. (1997). Role of nitric oxide and nitric oxide-independent relaxing factor in contraction and relaxation of rabbit blood vessels. *Eur. J. Pharmacol.,* **330,** 177–184.

GIERSE, J.K., HAUSER, S.D., CREELY, D.P., KOBOLDT, C., RANGWALA, S.H., ISAKSON, P.C. & SEIBERT, K. (1995). Expression and selective inhibition of the constitutive and inducible forms of human cyclo-oxygenase. *Biochem. J.,* **305,** 479–484.

HORI, M., SATO, K., SAKATA, K., OZAKI, H., TAKANO-OHMURO, H., TSUCHIYA, T., SUGI, H., KATO, I. & KARAKI, H. (1992). Receptor agonists induce myosin phosphorylation-dependent and phosphorylation-independent contractions in vascular smooth muscle. *J. Pharmacol. Exp. Ther.,* **261,** 506–512.

HUGHES, A.D. (1995). Increase in tone and intracellular Ca^{2+} in rabbit isolated ear artery by platelet-derived growth factor. *Br. J. Pharmacol.,* **114,** 138–142.

IKEDA, M., MORITA, C., MIZUNO, M., SADA, T., KOIKE, H. & KUROKAWA, K. (1997). PDGF-BB decreases systolic blood pressure through an increase in macrovascular compliance in rats. *Am. J. Physiol.,* **273,** H1719–H1726.

ITO, S., SUZUKI, S. & ITOH, T. (1993). Effects of a water-soluble forskolin derivative (NKH477) and a membrane-permeable cyclic AMP analogue on noradrenaline-induced Ca^{2+} mobilization in smooth muscle of rabbit mesenteric artery. *Br. J. Pharmacol.,* **110,** 1117–1125.

IWASAKI, T., NOTOYA, M., HAYASAKI-KAJIWARA, Y., SHIMAMURA, T., NAYA, N., NINOMIYA, M. & NAKAJIMA, M. (1999). Endothelium-independent vascular relaxation mediating ET_B receptor in rabbit mesenteric arteries. *Am. J. Physiol.,* **276,** H383–H390.

KHAN, S.A., MATHEWS, W.R. & MEISHERI, K.D. (1993). Role of calcium-activated K^+ channels in vasodilation induced by nitroglycerine, acetylcholine and nitric oxide. *J. Pharmacol. Exp. Ther.,* **267,** 1327–1335.

KOVALENKO, M., GAZIT, A., BOHMER, A., RORSMAN, C., RONNSTRAND, L., HELDIN, C.H., WALTENBERGER, J., BOHMER, F.D. & LEVITZKI, A. (1994). Selective platelet-derived growth factor receptor kinase blockers reverse sis-transformation. *Cancer Res.,* **54,** 6106–6114.

LEVITZKI, A. & GAZIT, A. (1995). Tyrosine kinase inhibition: An approach to drug development. *Science,* **267,** 1782–1788.

LI, J.Y. & KURIYAMA, H. (1993). Comparison of actions of endothelium-derived nitric oxide and sodium nitroprusside on mechanical responses evoked in aorta and mesenteric artery of the rabbit. *Gen. Pharmacol.,* **24,** 377–385.

MATTHEWS, J.S., MCWILLIAMS, P.J., KEY, B.J. & KEEN, M. (1995). Inhibition of prostacyclin release from cultured endothelial cells by nitrovasodilator drugs. *Biochem. Biophys. Acta.,* **1269,** 237–242.

OSANAI, T., FUJITA, N., FUJIWARA, N., NAKANO, T., TAKAHASHI, K., GUAN, W. & OKAMURA, K. (2000). Cross talk of shear-induced production of prostacyclin and nitric oxide in endothelial cells. *Am. J. Physiol.,* **278,** H233–H238.

OTTO, J.C. & SMITH, W.L. (1995). Prostaglandin endoperoxide synthase-1 and -2. *J. Lipid Mediat. Cell Signal.,* **12,** 139–156.

OZAKI, H., ABE, A., UEHIGASHI, Y., KINOSHITA, M., HORI, M., MITSUI-SAITO, M. & KARAKI, H. (1996). Effects of prostaglandin I_2 analog iloprost on cytoplasmic Ca^{2+} levels and muscle contraction in isolated guinea pig aorta. *Jpn. J. Pharmacol.,* **71,** 231–237.

PRITCHARD, JR. K.A., O'BANION, M.K., MIANO, J.M., VLASIC, N., BHATIA, U.G., YOUNG, D.A. & STEMERMAN, M.B. (1994). Induction of cyclooxygenase-2 in rat vascular smooth muscle cells in vitro and in vivo. *J. Biol. Chem:,* **269,** 8504–8509.

RIMARACHIN, J.A., JACOBSON, J.A., SZABO, P., MACLOUF, J., CREMINON, C. & WEKSLER, B.B. (1994). Regulation of cyclooxygenase-2 expression in aortic smooth muscle cells. *Arterioscler. Thromb.,* **14,** 1021–1031.

ROSS, R. (1993). The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature,* **362,** 801–809.

ROSS, R., RAINES, E.W. & BOWEN-POPE, D.F. (1986). The biology of platelet-derived growth factor. *Cell,* **46,** 155–169.

SAURO, M.D. & THOMAS, B. (1993). Tyrphostin attenuates platelet-derived growth factor-induced contraction in aortic smooth muscle through inhibition of protein tyrosine kinase(s). *J. Pharmacol. Exp. Ther.,* **267,** 1119–1125.

SMITH, W.L. (1986). Prostaglandin biosynthesis and its compartmention in vascular smooth muscle and endothelial cells. *Annu. Rev. Physiol.,* **48,** 251–262.

TAKASE, H., OEMAR, B.S., PECH, M. & LÜSCHER, T.F. (1999). Platelet-derived growth factor-induced vasodilatation in mesenteric resistance arteries by nitric oxide: blunted response in spontaneous hypertension. *J. Cardiovasc. Pharmacol.,* **33,** 223–228.

VINALS, M., MARTINEZ-GONZALEZ, J., BADIMON, J.J. & BADIMON, L. (1997). HDL-induced prostacyclin release in smooth muscle cells is dependent on cyclooxygenase-2 (Cox-2). *Arterioscler. Thromb.,* **17,** 3481–3488.

YAMAWAKI, H., SATO, K., HORI, M., OZAKI, H., NAKAMURA, S-I., NAKAYAMA, H., DOI, K. & KARAKI, H. (2000). Morphological and functional changes of rabbit mesenteric artery cultured with fetal bovine serum. *Life Sci.,* **67,** 807–820.

(Received August 21, 2000
Revised October 12, 2000
Accepted October 12, 2000)